

Claims

1-4. (Canceled)

5. (Currently amended) An isolated nucleic acid molecule encoding a substantially purified RFX4_v3 polypeptide, wherein the polypeptide comprises:

a) an amino acid sequence at least 70% identical to an amino acid sequence set forth as SEQ ID NO: 8;

b) a conservative variant of the amino acid sequence set forth as SEQ ID NO: 8; or

c) the amino acid sequence set forth as SEQ ID NO: 8,
wherein the polypeptide has RFX4_v3 activity, and the N-terminus of the polypeptide is at least 90% identical to residues 1-14 of SEQ ID NO: 8~~the polypeptide of claim 2.~~

6. (Original) The nucleic acid of claim 5, wherein the nucleic acid molecule comprises: a nucleic acid sequence at least 70% identical to the nucleic acid sequence set forth as SEQ ID NO: 37.

7. (Withdrawn) The nucleic acid of claim 6, wherein the nucleic acid sequence is at least 90% identical to SEQ ID NO: 38 or SEQ ID NO: 39.

8. (Original) The nucleic acid of claim 6, wherein the nucleic acid sequence is at least 90% identical to SEQ ID NO: 37.

9. (Original) The nucleic acid sequence of claim 5, wherein the nucleic acid sequence is operably linked to a heterologous promoter.

10. (Original) The nucleic acid sequence of claim 5, wherein the heterologous promoter comprises SEQ ID NO: 11 or SEQ ID NO: 12.

11. (Original) A vector comprising the nucleic acid of claim 5.

12. (Currently amended) An in vitro host cell transformed with the vector of claim 11.

13. (Currently amended) The in vitro host cell of claim 12, wherein the host cell is a plant cell, an animal cell, or a prokaryotic cell.

14. (Withdrawn) A composition comprising the polypeptide of claim 2.

15. (Currently amended) An isolated nucleic acid molecule that hybridizes under conditions of low stringency to a polynucleotide ~~comprising~~ consisting of nucleotides 1-42 of a nucleic acid sequence selected from the group consisting of SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39, wherein the isolated nucleic acid molecule comprises at least 15 nucleotides.

16. (Previously presented) The isolated nucleic acid molecule of claim 15, wherein the isolated nucleic acid molecule hybridizes under conditions of high stringency to the polynucleotide.

17. (Previously presented) The nucleic acid of claim 15, wherein the isolated nucleic acid molecule encodes a RFX4_v3 polypeptide.

18. (Previously presented) The nucleic acid of claim 17, wherein the RFX4_v3 polypeptide comprises SEQ ID NO: 6, SEQ ID NO: 8, or SEQ ID NO: 10.

19. (Original) A vector comprising the nucleic acid of claim 15.

20. (Currently amended) An in vitro host cell transformed with the vector of claim 19.

21. (Currently amended) The in vitro host cell of claim 20, wherein the host cell is a plant cell, an animal cell, or a prokaryotic cell.

22-24. (Canceled)

25. (Currently amended) A method for producing a variant RFX4_v3 ~~of the~~ polypeptide ~~of claim 2~~, wherein the method comprises:

mutagenizing a wild-type nucleic acid sequence as set forth in SEQ ID NO: 37, SEQ ID NO: 38, or SEQ ID NO: 39;

expressing the mutagenized nucleic acid sequence; and

screening the variant for a RFX4_v3 activity to identify the variant of the RFX4_v3 polypeptide, wherein the RFX4_v3 polypeptide comprises:

a) an amino acid sequence at least 70% identical to an amino acid sequence set forth as SEQ ID NO: 8;

b) a conservative variant of the amino acid sequence set forth as SEQ ID NO: 8; or

c) the amino acid sequence set forth as SEQ ID NO: 8,
wherein the polypeptide has RFX4_v3 activity, and the N-terminus of the polypeptide is at least 90% identical to residues 1-14 of SEQ ID NO: 8.

26. (Previously presented) A composition comprising a nucleic acid molecule that inhibits the binding of the polynucleotide of claim 15 to its complementary sequence.

27. (Previously presented) The isolated nucleic acid of claim 15, wherein the isolated nucleic acid hybridizes under stringent conditions to the polynucleotide comprising nucleotides 1-42 of SEQ ID NO: 37.

28. (Withdrawn and currently amended) A method for detecting a nucleic acid molecule in a biological sample, comprising:

hybridizing a polynucleotide to the nucleic acid molecule of claim 5 to produce a hybridization complex, ~~wherein the nucleic acid molecule encodes the RFX4_v3 polypeptide of claim 2 and~~ wherein the polynucleotide hybridizes to nucleotides 1-42 of SEQ ID NO: 37, SEQ ID NO: 38, or SEQ ID NO: 39;

detecting the hybridization complex, wherein the presence of the hybridization complex indicates the presence of the nucleic acid molecule encoding RFX4_v3 in the biological sample, thereby detecting the nucleic acid molecule in the biological sample.

29. (Withdrawn) The method of claim 28, wherein the polynucleotide hybridizes to SEQ ID NO: 37.

30. (Withdrawn) The method of claim 28, further comprising amplifying the nucleic acid molecule prior to hybridizing with the polynucleotide.

31. (Withdrawn and currently amended) A method of identifying a subject at risk of developing RFX4_v3 linked hydrocephalus, comprising detecting in the subject a mutation in the RFX4_v3 polypeptide of claim 2 or a mutation in a RFX4_v3 nucleotide sequence encoding the RFX_v3 polypeptide nucleic acid molecule of claim 5, wherein the mutation in the nucleotide sequence nucleic acid molecule alters the RFX4_v3 polypeptide, thereby identifying a subject at risk of developing RFX4_v3 linked hydrocephalus.

32. (canceled)

33. (Withdrawn and currently amended) The method of claim 31, wherein detecting the mutation in the RFX4_v3 nucleotide sequence nucleic acid molecule comprises performing a hybridization analysis with a nucleic acid probe that detects the mutation in the RFX4_v3 nucleic acid sequence molecule.

34. (Withdrawn) The method of claim 31, wherein detecting the mutation comprises identifying an individual carrying a mutated RFX4_v3 allele, wherein the method comprises: providing a nucleic acid from the subject, wherein the nucleic acid comprises a RFX4_v3 allele; and

detecting a mutation in the nucleic acid that results in phenotypic expression of congenital hydrocephalus.

35. (Withdrawn) The method of claim 34, wherein the mutation is in the RFX4_v3 allele.
36. (Withdrawn) The method of claim 31, wherein the method comprises detecting the mutation in the RFX4_v3 polypeptide.
37. (Withdrawn) The method of claim 36, wherein the method comprises detecting an abnormality in expression of the RFX4_v3 polypeptide.
38. (Withdrawn) The method of claim 37, wherein the method detects a reduced expression of the RFX4_v3 polypeptide.
39. (Withdrawn) The method of claim 36, wherein the method comprises providing a polypeptide from the subject, and detecting a mutation in the sequence encoding the polypeptide, wherein the polypeptide comprises the RFX4_v3 polypeptide and wherein the mutation results in phenotypic expression of congenital hydrocephalus.
40. (Withdrawn) The method of claim 31, comprising obtaining a biological sample from the subject, and detecting in the biological sample the mutation in the RFX4_v3 polypeptide or in the RFX4_v3 nucleotide sequence.
41. (Withdrawn) The method of claim 40, wherein the biological sample comprises blood, amniotic fluid, plasma, or cerebral spinal fluid.
42. (canceled).
43. (Withdrawn) The method of claim 38, wherein detecting the reduced expression of the RFX4_v3 polypeptide comprises using RFX4_v1 specific antibodies.
44. (Withdrawn) A kit for determining if a subject is a carrier of a mutated RFX4_v3 gene, wherein the kit comprises:
a reagent that specifically detects a mutation in a RFX4_v3 allele, and

instructions for determining whether the subject is at increased risk of expressing congenital hydrocephalus if the reagent specifically detects the mutation.

45. (Withdrawn) The kit of claim 44, wherein the reagent comprises a nucleic acid probe that specifically hybridizes under stringent conditions to a nucleic acid sequence of SEQ ID NO: 37, SEQ ID NO: 38 or SEQ ID NO: 39.

46. (Withdrawn) The kit of claim 44, wherein the reagent comprises an antibody that specifically binds the protein expressed by the RFX4_v3 allele.

47-48. (canceled)

49. (Withdrawn) An antibody that specifically binds the polypeptide of claim 2.

50. (Withdrawn) A method for generating a non-human transgenic animal with a knockout for the RFX4_v3 gene, wherein the method comprises disrupting an RFX4_v3 transcript, the disruption being sufficient to produce hydrocephalus in the transgenic animal.

51. (Withdrawn) The method of claim 50, wherein the non-human transgenic animal is a mouse.

52. (Withdrawn) The method of claim 50, wherein disrupting a RFX4_v3 transcript comprises:
deleting or substituting any portion of the RFX4_v3 transcript,
inserting an exogenous gene into the RFX4_v3 transcript, or
any combination thereof.

53. (Withdrawn) The method of claim 50, wherein disrupting the RFX4_v3 transcript comprises crossing one non-human transgenic animal with a second non-human transgenic animal.

54. (Withdrawn) A transgenic mouse whose somatic and germ cells comprise a disrupted endogenous RFX4_v3 gene, the disruption being sufficient to produce an increased susceptibility to developing congenital hydrocephalus.

55. (Withdrawn) The transgenic mouse of claim 54, wherein the disrupted gene is introduced into the mouse of an ancestor of the mouse at an embryonic stage, wherein the mouse, if homozygous for the disrupted gene, does not reproduce.

56. (Withdrawn) The transgenic mouse of claim 54, wherein the disruption is an insertion within the RFX4_v3 gene.

57. (Withdrawn) The composition of claim 54, wherein the disruption is a deletion or substitution within the RFX4_v3 gene.

58. (Withdrawn) A method for screening compounds for the ability to alter RFX4_v3 activity, wherein the method comprises:

- a) providing:
 - i) a first polypeptide sequence comprising at least a portion of the polypeptide of claim 2,
 - ii) a second polypeptide sequence comprising at least a portion of a protein known to interact with RFX4_v3, and
 - iii) one or more test compounds; and
- b) combining in any order (i), (ii), and (iii) under conditions such that the first polypeptide sequence, the second polypeptide sequence, and the test compound interact; and
- c) detecting the presence or absence of an interaction between the first polypeptide sequence and the second polypeptide sequence.

59. (Withdrawn) A pharmaceutical composition, comprising:

- a) a therapeutically effective amount of the polypeptide of claim 2, a nucleic acid sequence encoding the polypeptide, or a therapeutically effective variant or portion thereof; and

b) a pharmaceutically acceptable carrier.

60. (canceled).

61. (Withdrawn) A method of treating congenital hydrocephalus in a subject, comprising administering to the subject a therapeutically effective amount of an agent to the subject.

62. (Withdrawn) The method of claim 61, wherein the method comprises administering exogenous RFX4_v3 polypeptide to the subject.

63. (Withdrawn) The method of claim 61, wherein the method comprises increasing expression of RFX4_v3 polypeptide in the subject.

64. (Withdrawn) The method of claim 63, wherein the method comprises introducing into the subject a vector that expresses the RFX4_v3 polypeptide in the subject.

65. (New) The isolated nucleic acid molecule of claim 5, wherein the polypeptide comprises an amino acid sequence at least 80% identical to an amino acid sequence set forth as SEQ ID NO: 8.

66. (New) The isolated nucleic acid molecule of claim 65, wherein the polypeptide comprises an amino acid sequence at least 85% identical to an amino acid sequence set forth as SEQ ID NO: 8.

67. (New) The isolated nucleic acid molecule of claim 65, wherein the polypeptide comprises an amino acid sequence at least 90% identical to an amino acid sequence set forth as SEQ ID NO: 8.

68. (New) The isolated nucleic acid molecule of claim 5, wherein the RFX4_v3 activity comprises inhibiting the phenotypic expression of congenital hydrocephalus.

69. (New) The isolated nucleic acid molecule of claim 5, wherein the activity is the ability to bind to RFX4_v3 specific antibodies.

70. (New) The isolated nucleic acid molecule of claim 5, wherein the polypeptide comprises the amino acid residues set forth in SEQ ID NO: 33, SEQ ID NO: 34, or SEQ ID NO: 35.

71. (New) The nucleic acid of claim 6, wherein the nucleic acid molecule comprises: a nucleic acid sequence at least 80% identical to the nucleic acid sequence set forth as SEQ ID NO: 37.

72. (New) An isolated nucleic acid molecule that hybridizes under conditions of low stringency to a polynucleotide comprising at least 20 contiguous nucleotides in the region between nucleotides 1 and 42 of a nucleic acid sequence selected from the group consisting of SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39.

73. (New) The isolated nucleic acid molecule of claim 72, wherein the isolated nucleic acid molecule hybridizes under conditions of high stringency to the polynucleotide.